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Insulin clearance as the major player in the hyperinsulinaemia of black African men without diabetes

Short title: Insulin clearance, hepatic fat and insulin sensitivity in black and white men

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ABSTRACT

AIMS: Despite their low levels of ectopic fat accumulation, populations of African ancestry exhibit hyperinsulinaemia and increased metabolic risk. We aimed to investigate relationships between insulin clearance, insulin secretion, hepatic fat accumulation and insulin sensitivity in black African (BA) and white European (WE) men.

METHODS: 23 BA and 23 WE men with normal glucose tolerance, matched for age and body mass index, underwent a hyperglycaemic clamp to measure insulin secretion and clearance; hyperinsulinaemic-euglycaemic clamp with stable glucose isotope infusion to measure whole-body and hepatic-specific insulin sensitivity; and magnetic resonance imaging to quantify intrahepatic lipid (IHL).

RESULTS: BA men had higher glucose-stimulated peripheral insulin levels (48.1 (35.5, 65.2) x 10^3 *vs* 29.9 (23.3, 38.4) x10^3 pmol L^{-1} x min, p=0.017) and lower endogeneous insulin clearance (771.6 (227.8) *vs* 1381 (534.3) mL m^{-2} BSA min $^{-1}$, p<0.001) compared with WE men. There were no ethnic differences in beta cell insulin secretion or beta cell responsivity to glucose, even after adjustment for prevailing insulin sensitivity. In WE men, endogenous insulin clearance was correlated with whole-body insulin sensitivity (r=0.691, p=0.001) and inversely correlated with IHL (r= -0.674, p=0.001). These associations were not found in BA men.

CONCLUSIONS: While normally glucose tolerant BA men have similar insulin secretory responses to their WE counterparts, they have markedly lower insulin clearance, which does not appear to be explained by either insulin resistance or hepatic fat accumulation. Low insulin clearance may be the primary mechanism of hyperinsulinaemia in populations of African origin.

KEY WORDS: African, ethnicity, insulin clearance, insulin secretion, intrahepatic lipid

ABBREVIATIONS

BA: Black African

HFF: Hepatic Fat Fraction

EGP: Endogenous glucose production

FFM: Fat-free mass

iAUC: Incremental area under the curve

IHL: Intrahepatic lipid

ISR: Insulin secretion rate

MCRI: Metabolic clearance rate of insulin

NEFA: Non-esterified fatty acids

OGTT: Oral glucose tolerance test

WE: White European

INTRODUCTION

The multifaceted pathophysiology of type 2 diabetes (T2D) includes peripheral and hepatic insulin resistance, reduced insulin clearance and beta-cell dysfunction. Insulin secreted by the pancreatic beta cell is delivered directly via the portal vein to the liver, where the majority of endogenous insulin clearance occurs [1]. The predominant mechanism of hepatic insulin clearance involves insulin binding to its receptor on the hepatocyte surface, with endocytosis and internalisation of the insulin-receptor complex and subsequent degradation [2]. Therefore, hepatic insulin clearance is an integral part of insulin's action on the liver, with greater hepatic insulin sensitivity associated with greater clearance [3]. Intrahepatic lipid (IHL) plays a key role in glucose/insulin dysregulation; while the mechanisms are not fully understood, the accumulation of lipotoxic mediators has been found to inhibit insulin receptor activation [4]. In this way, accumulation of IHL is believed to drive impairments in both insulin clearance and hepatic insulin sensitivity [5-8].

Populations of black African (BA) ethnicity suffer a disproportionately elevated risk of T2D [9, 10], yet they are relatively protected from ectopic fat deposition and exhibit lower IHL relative to other ethnicities (the so-called "African paradox") [11]. Distinctive features of insulin dynamics are well-documented in populations of African ethnicity [12], with an exaggerated insulin response to glucose in BA subjects demonstrated across a spectrum of glucose tolerance [13-17]. An important contributor to this phenomenon is the relatively low insulin clearance of BA populations, which has been consistently recognised [18-22]. Reductions in insulin clearance appear to be a predictor of T2D in this ethnic group [23] and may be associated with increased markers of inflammation [24].

We have previously shown that fasting hepatic insulin resistance in BA men with early T2D appears to be independent of IHL [25] and that there are ethnic differences in the relationship between ectopic fat accumulation and insulin sensitivity [26]. This has led us to hypothesise that the role of IHL in insulin clearance may differ by ethnicity. To our knowledge, this is the first study to examine the impact of BA ethnicity on relationships between insulin clearance, insulin sensitivity and hepatic fat in adult men of normal glucose tolerance.

METHODS

Study Design

The data were collected as part of "Soul-Deep II", a cross-sectional study of the development of type 2 diabetes in men resident in South London from two ethnic groups, white European (WE) and black (West) African (BA). Metabolic assessments were performed at the Clinical Research Facility, King's College Hospital, London, UK, while MRI imaging took place at Guy's Hospital, London, UK. The study was approved by the London Bridge National Research Ethics Committee (15/LO/1121). Recruitment of subjects and data collection took place between April 2016 and May 2018. Recruitment was carried out through advertising in the local press and via South London primary care practices. All subjects provided written informed consent prior to the study.

Subjects

Eligible subjects were male, aged 18-65 years, of either white European (WE) or black (West) African (BA) ethnicity. Ethnicity was self-declared and confirmed by grandparental birthplace. Eligible WE subjects had 4 WE grandparents with at least two of these from North West European countries as defined by the United Nations Statistics Division (UNSD) [27]. Eligible BA subjects had 4 BA grandparents from West African countries as defined by UNSD.

Subjects were invited to a screening assessment at the Clinical Research Facility at King's College Hospital, following a 10 hour fast, in order to undertake a screening questionnaire, anthropometric measurements and a 2 hour, 75g oral glucose tolerance test (OGTT). Eligible subjects were normal glucose tolerant according to World Health Organisation criteria [28].

Exclusion criteria were: a diagnosis of diabetes; treatment with oral hypoglycaemic agents, insulin, systemic steroids or beta blockers; any condition or medication considered by the investigators to have substantial impact on the study protocol or outcomes; serum creatinine of >150 µmol/l; serum alanine transaminase level >2.5 fold above the upper limit of the reference range; sickle cell disease (trait permitted). Participants were instructed to refrain from 1) strenuous physical activity for 48-hours 2) alcohol consumption for 24-hours and 3) food and drink (other than water) for at least 10 hours prior to the study visits.

Hyperglycaemic clamp assessment of first- and second- phase insulin secretory function and insulin clearance

Following an overnight fast, participants were admitted to the Clinical Research Facility and weighed in light clothing. A cannula was inserted into the antecubital fossa vein of the non-dominant arm for administration of the glucose infusion and a second cannula inserted retrogradely into the dorsum of the contralateral hand for blood sampling. The sampling hand was placed in a hand-warming unit at 55 ºC in order to achieve arterialised venous blood. A primed, variable rate intravenous infusion of 20% (wt/vol) dextrose was administered for 120 minutes to achieve square-wave hyperglycaemia with a plasma glucose concentration of 6.9 mmol/L above baseline, according to the protocol of DeFronzo et al [29]. Glucose, insulin, and C-peptide concentrations were measured at fasting (-20, -10, 0 minutes) and at 2, 4, 6, 8, 10, 15, 20, 30, 40, 50, 60, 75, 90, 105, and 120 minutes.

Hyperinsulinaemic-euglycaemic clamp assessment of insulin sensitivity

The full methodology has been described [26]. In brief, a two-step hyperinsulinaemic–euglycaemic clamp with a stable glucose isotope infusion was used to assess whole-body and hepatic-specific insulin sensitivity. Participants were admitted to the Clinical Research Facility following an overnight fast and weighed in light clothing. During the basal phase, a primed

(2.0 mg/kg), continuous infusion (0.02 mg kg^{-1} min^{-1}) of [6,6 $^2H_2$]-glucose (CK Gases, Cambridgeshire, UK) was initiated at -120 minutes. Blood samples were taken at -30, -20, -10 and 0 minutes for basal assessments. The clamp began at 0 minutes with a primed continuous insulin infusion (Actrapid, Novo Nordisk, Bagsvaerd, Denmark) bound to albumin at a rate of 10 mU m^{-2} BSA min^{-1} for 2 hours (low dose insulin phase) for assessment of hepatic insulin sensitivity. For the final 2 hours, the insulin infusion rate was re-primed and increased to 40 mU m^{-2} BSA min^{-1} (high dose insulin phase) for assessment of whole-body insulin sensitivity. Euglycaemia (5.0 mmol/l) was achieved using variable rate 20% (wt/vol) dextrose enriched with [6,6 $^2H_2$]-glucose (8 mg/g glucose) to maintain a constant tracer-to-tracee ratio. Blood was drawn at 30, 60, 90, 100, 110, 120, 150, 180, 210, 220, 230 and 240 minutes for the assessment of plasma glucose concentration, isotopic enrichment and insulin concentration.

Magnetic Resonance Imaging assessment of IHL

The full imaging protocol has been reported [30]. In brief, a Dixon-based MRI sequence was used on a 1.5 Tesla Siemens scanner to obtain images from the neck to the knee (excluding arms). 384 contiguous, axial T1-weighted gradient-echo images with a slice thickness of 3mm were acquired, from which fat and water images were produced as part of the Dixon sequence. MRI data were analysed using the open source image analysis software HOROS V 1.1.7 (www.horosproject.org; accessed 21/10/2017) by a single, blinded analyst (OH).

In each participant, two abdominal MRI images approximately 30mm apart were selected, representing the superior and inferior sections of the liver. In each pair of water and fat MRI images, 4 circular regions of interest (ROIs) in identical positions were placed within the liver tissue. The hepatic fat fraction (HFF) was quantified in each ROI by using the formula %HFF = (F/(F+W)) *100 where F is the pixel signal intensity of the fat image and W is the pixel signal intensity of the water image. Intrahepatic lipid (IHL) was calculated as the mean of all 8 ROIs.

Biochemical analyses

Plasma glucose concentrations were determined at the bedside using an automated glucose analyser (Yellow Spring Instruments, 2300 STAT Glucose Analyzer, Ohio, USA). Plasma insulin concentrations were determined by immunoassay using chemiluminescent technology (ADVIA Centaur System, Siemens Healthcare Ltd. Camberly, UK). Plasma C-peptide concentrations were determined by radioimmunoassay (Millipore Ltd, Hertfordshire, UK). Plasma glucose isotope enrichments were measured by gas chromatography-mass spectrometry on an Agilent GCMS 5975C MSD (Agilent Technologies, Wokingham, UK).

Calculations

Whole-body insulin sensitivity was quantified using the M value (mg/kg FFM min^{-1}) measured during the final 30 min of the high-dose insulin phase of the clamp, calculated as total glucose disposal corrected for deviations in plasma glucose concentration [26]. Whole-body insulin sensitivity was also expressed as M/I, the M value corrected for the steady state insulin concentration during the last 30 minutes of the clamp (mg kg^{-1} FFM min^{-1})/ (pmol L^{-1}).

Steele's non-steady-state equations, modified for stable isotopes, were used to determine total glucose rate of appearance, Ra (μmol kg^{-1} FFM min^{-1}) [31]. Endogenous glucose production (EGP) was calculated by subtracting exogenous glucose infusion rate from total glucose Ra. Hepatic insulin sensitivity was expressed as the percentage suppression of EGP from basal to the final 30 minutes of the clamp (% suppression of EGP).

The clearance rate of the exogenously administered insulin infusion during the hyperinsulinaemic-euglycaemic clamp (metabolic clearance rate of insulin, or MCRI) was

197 calculated as the insulin infusion rate divided by the insulin concentration during the steady
198 state period in the final 30 minutes of the clamp (mL m^{-2} BSA min^{-1}).
199
200 The incremental areas under the curve (iAUC) were calculated using the trapezoid rule for C-
201 peptide, insulin and glucose. Classical indices of first- and second-phase insulin secretion
202 during the hyperglycaemic clamp were determined by calculating the iAUC for C-peptide for
203 0 to 10 minutes and 10 to 120 minutes respectively.
204
205 Parameters of beta cell function were obtained by modelling the glucose and C-peptide curves
206 during the hyperglycaemic clamp using published methods [32, 33]. Model assessments were
207 carried out using SAAM-II 1.2 software (SAAM Institute, Seattle, Washington). The main
208 outputs of the model are: pre-hepatic endogenous insulin secretion (expressed as area under
209 the curve of insulin secretion rate over 120 minutes, AUC_{ISR}); beta cell glucose sensitivity of
210 first-phase secretion (σ1), expressed as the amount of insulin secreted in response to a rate of
211 increase in glucose of 1 mmol/L between time 0 and 1 minute of the study, in (pmol m^{-2}
212 BSA)/(mmol L^{-1} min^{-1}), beta cell glucose sensitivity of second-phase secretion (σ2), expressed
213 as the steady-state insulin secretion rate in response to a step increase in glucose of 1 mmol/L
214 above baseline, in (pmol min^{-1} m^2)/(mmol L^{-1}).
215
216 During the hyperglycaemic clamp, average (endogenous) insulin clearance was calculated
217 according to the following formula [33]:

$$Clearance_{Ins} = \frac{AUC_{ISR}}{AUC_I + (I_{Final} - I_{Basal}) \cdot MRT_{Ins}}$$

218
219 where AUC_{ISR} is the area under the curve of insulin secretion rate, AUC_I is the area under the
220 curve of insulin concentration, I_{Final} is insulin concentration at the end of the study, I_{Basal} is

insulin concentration at the beginning of the study, and MRT_{Ins} is the mean residence time of insulin, which was assumed to be 18 minutes as reported in Navalesi *et al* [34].

Statistical analysis

Log-transformation was used on skewed variables that showed a significant deviation from normality to achieve a normal distribution prior to the use of parametric tests. Data are expressed as means (SD) for non-transformed data and geometric mean (95% confidence intervals) for log-transformed data. Significance of differences in variables between the two ethnic groups were made using independent sample Student's *t* test. The strength of associations between variables of interest was assessed using Pearson's correlation. The ethnic differences in relationships between endogenous insulin clearance and whole-body insulin sensitivity, hepatic insulin sensitivity, intrahepatic lipid and MCRI, were examined by fitting a regression model between the pairs of variables with an interaction term for ethnicity. Prior to running the regression models, collinearity diagnostics were performed for the whole cohort for insulin clearance with ethnicity, hepatic fat (IHL), insulin sensitivity (M value) and insulin secretion (AUC_ISR). The VIFs for these factors were used to exclude multicollinearity. An ANCOVA was used, with insulin secretion (AUC_{ISR}), intrahepatic lipid (IHL), hepatic insulin sensitivity (% suppression of EGP) and whole-body insulin sensitivity (M value) as co-variates, to investigate ethnic differences in average endogenous insulin clearance. An ANCOVA was used with whole-body insulin sensitivity (M value) as a co-variate, to investigate ethnic differences in endogenous insulin secretion. Missing data were excluded pairwise for all analyses; in the case of the correlation analysis between IHL and insulin clearance, this led to skewing of the IHL data which was therefore log-transformed for this analysis. All analyses were conducted with SPSS version 25.0 and p values< 0.05 were considered statistically significant.

RESULTS

Participant characteristics

The characteristics of the 23 BA and 23 WE men are presented in Table 1. The two ethnic groups were well-matched for age, weight and BMI and showed no difference in HbA_{1c}, blood pressure or fasting glucose (Table 1). The BA men had significantly lower fasting triglyceride levels (Table 1).

Beta cell insulin secretory function

There were no ethnic differences in fasting C-peptide or fasting insulin (Table 2). By design, there was no difference in "clamped" glucose during the hyperglycaemic clamp (BA= 12.1 (0.65) *vs* WE = 12.0 (0.63) mmol/L, p= 0.635).

Mean peripheral insulin levels were approximately 1.5-fold higher during both first and second phase of the hyperglycaemic clamp in BA compared with WE men (Figure 1a), while there were no ethnic differences in C-peptide response (Table 2, Figure 1b), endogenous beta cell insulin secretion (AUC_{ISR}) (Table 2) or sensitivity of the beta cell to glucose during first or second phase insulin secretion, σ^1 and σ^2 (Table 2). This remained the case after measures of beta cell insulin secretion were adjusted for whole-body insulin sensitivity (p = 0.512).

Intrahepatic lipid and insulin sensitivity

Data on IHL, whole body and hepatic insulin sensitivity, as previously reported by our group [26, 30] showed IHL was lower in BA men, while there were no ethnic differences in hepatic insulin sensitivity or whole-body insulin sensitivity by either M value or M/I (included in Table 2 for reference).

Insulin clearance

Average endogenous insulin clearance was almost 50% lower in BA compared with WE men during the hyperglycaemic clamp (Table 2). The ethnic difference remained significant after adjusting for whole-body insulin sensitivity (M value), endogenous insulin secretion (AUC_{ISR}), intrahepatic lipid (IHL) and hepatic insulin sensitivity (% suppression of EGP) ($p < 0.001$). Clearance of exogenous insulin as determined by MCRI was also lower in BA compared with WE men (Table 2).

Relationships between endogenous insulin clearance and intrahepatic lipid, insulin sensitivity and insulin secretion

In WE men, endogenous insulin clearance was correlated with whole-body (Figure 2a & 2b) and hepatic insulin sensitivity (Figure 2c) and with MCRI (Figure 2d), while it was inversely correlated with IHL (Figure 2e). These relationships were not found in the BA men (figure 2 a-e).

In multiple regression analysis, an ethnicity interaction was found in the relationship between endogenous insulin clearance and whole-body insulin sensitivity (Figure 2a; $p\text{-}_{interaction} = 0.022$). An ethnicity interaction was also found in the relationship between the measurements of endogenous insulin clearance and MCRI (Figure 2d; $p\text{-}_{interaction} = 0.021$). A trend was found for an ethnicity interaction between endogenous insulin clearance and hepatic insulin sensitivity (Figure 2c; $p\text{-}_{interaction}=0.057$). No ethnicity interaction was found between insulin clearance and IHL.

DISCUSSION

This study comprises a comprehensive investigation of insulin clearance and its relationships with hepatic fat and insulin sensitivity in white European and black African men with normal glucose tolerance. To our knowledge, it is the first to demonstrate that the markedly low insulin clearance of an African origin population may occur in the absence of insulin resistance. While the classical paradigm suggests that increased insulin resistance drives the excess diabetes risk in BA populations, these findings contribute to a newly emerging (and as yet, controversial) paradigm, which proposes that impairments in insulin clearance are the primary aetiological mechanism of glucose intolerance in this ethnic group [35, 36].

In this study, the response to intravenous glucose in the BA men was characterised by a pronounced hyperinsulinaemia compared with that of the WE men (Figure 1). While this is well-documented in the literature, it has previously been described as a compensatory response by the beta cell to increased insulin resistance and/or a consequence of "upregulated" beta cell function [14, 18, 37-39]. By contrast, in our study population we found that there were no ethnic differences in total beta cell insulin secretion, corroborating the findings of the Federal Women's Study [22] and developing an evidence base which disputes the argument that people of African ancestry typically exhibit insulin hypersecretion.

We acknowledge that much of the literature, including the Federal Women's study [22], report greater first phase beta cell responsivity in African-ancestry groups [38] whereas we found no ethnic differences in either first or second phase beta cell responsivity to glucose. Our results may differ as this is one of the few studies to comprise exclusively of adult men, rather than children/adolescents [18, 19, 37, 38, 40] or all-female cohorts [39, 41-45]. Two other adult non-diabetic all-male studies have examined ethnic differences in insulin responses [46, 47];

neither found increased insulin secretion in the African-ancestry subjects compared with white subjects, consistent with our findings. It is worth considering that the paucity of male participants in this area of the literature may have led to an overestimation of ethnic differences in insulin secretory response [48].

We found that the peripheral hyperinsulinaemia of our BA population was due to the ethnic difference in insulin clearance; furthermore, this difference persisted after adjustment for insulin sensitivity, hepatic fat content and insulin secretion rate. Whilst insulin clearance was associated with IHL and whole body and hepatic insulin sensitivity in WE men, these associations were absent in the BA men. Insulin clearance is a highly variable process which operates under the influence of multiple physiological factors. The predominant cellular mechanism is receptor-mediated uptake and therefore a correlation is typically seen between insulin sensitivity and insulin clearance [49, 50]. Our data in the WE men are in keeping with these expected relationships. In the BA men, where such relationships are not observed, we may postulate that such mechanisms operate differently between the ethnic groups, or with different dose-responses.

This leads us to propose that endogenous insulin clearance in BA may be determined by additional factors which are independent of insulin sensitivity, e.g. in the pathways involved in post-receptor insulin metabolism. This assertion is supported by recent evidence from a study investigating ethnic differences in the expression and activity of hepatic insulin-degrading enzyme (IDE) and carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM-1) between African-Americans and non-Hispanic White Americans, which reported lower IDE activity in African-Americans [51]. While reduced insulin clearance is widely regarded as an early response to insulin resistance [52, 53], it is also possible that changes in insulin clearance

347 are not only a compensatory mechanism but also a primary determinant of peripheral insulin
348 levels [36, 54] in BA populations.
349
350 Low insulin clearance has been demonstrated in African American women [22], in pre-pubertal
351 African-American children [18, 21, 55], in indigenous adult Ghanaians [56] and, with this
352 study, in black West African men living in the UK. The finding of this distinctive physiological
353 characteristic in diverse populations of African ancestry is suggestive of a genetic rather than
354 environmentally mediated mechanism. The strong heritability of insulin clearance has been
355 demonstrated in a Hispanic population [57] and this may also be the case in other ethnic groups.
356 Molecular mechanisms that warrant further exploration include potential ethnic differences in
357 inflammatory activity [24], the expression and activity of insulin-degrading enzyme [51], the
358 liver-adiponectin pathway [58], or in liver CEACAM-1 expression/activity [2].
359
360 We note that clearance of exogenously administered insulin (as determined by the MCRI) is
361 also lower in BA compared to WE, although the difference is not as marked as for endogenous
362 insulin clearance. This may be expected, because while only endogenously secreted insulin
363 undergoes first pass hepatic metabolism, exogenously administered insulin also undergoes both
364 hepatic and extra-hepatic clearance (with around 60% of peripherally administered insulin
365 thought to be cleared by the liver [59]).
366
367 Importantly, while MCRI is closely correlated with endogenous insulin clearance in WE, this
368 is not the case in BA and ethnicity has a significant impact on the relationship between the two
369 measures. We note that models have shown that while hepatic insulin clearance is lower in
370 black ethnic groups compared with whites, extra-hepatic clearance is similar [20, 21] and that
371 hepatic and extra-hepatic insulin clearance are differentially regulated [60]. As hepatic insulin

clearance contributes in greater proportion to endogenous compared with exogenous insulin clearance, this may explain why the MCRI does not reflect endogenous insulin clearance in the black African men. These finding have implications for the use of MCRI as a measure of insulin clearance in black ethnic groups.

The strengths of this study include the well-matched ethnic groups and the use of rigorous, gold-standard methods of metabolic analysis. Unlike some previous ethnic comparison studies [48], the subject groups were tightly characterised; of single sex with metabolic status confirmed by OGTT.

In terms of limitations, the study is cross-sectional and is only able to recognise the presence or absence of associations, between hepatic fat, insulin sensitivity and insulin clearance. Only longitudinal measures would be able to determine the true dynamics of these mechanisms and determine causality. The measure of average endogenous insulin clearance does not enable differentiation between hepatic and extra-hepatic insulin clearance, although it has been shown that approximately 80% of endogenous insulin is cleared by the liver [1]. Furthermore, the influence of gut-modulated insulin secretion and clearance was not assessed. There is evidence that the incretin hormones reduce post-prandial insulin clearance [61-63] and ethnic differences in incretin hormones have been recognised [33, 64, 65], albeit the data are inconsistent. However, it is not clear whether incretins contribute to ethnic differences in insulin clearance, which would be an important line of enquiry for future investigations. We also acknowledge that the failure to find an association between IHL and insulin clearance in the BA men does not mean that an association does not exist. Our sample size is comparable with other studies in the literature, but we may not be powered to reliably detect such associations. While the narrow range in IHL among our black participants also may have hindered our ability to detect

a linear correlation between IHL and insulin clearance, the range in IHL that we observed is reflective of IHL in black populations, as reported in a large epidemiological study [66] . In data we have previously reported from an obese diabetic population [25], we did find a trend to an inverse relationship between insulin clearance and intrahepatic lipid in the BA participants, suggesting that there may be a threshold mechanism at play and that IHL may not have a significant role in the determination of insulin clearance until higher levels of accumulation have occurred.

The study comprises male subjects only and therefore may not be generalisable to women; however, as the majority of the work in this area has been carried out in African ancestry females [48], this cohort offers a valuable addition to the field.

In conclusion, this study demonstrates low insulin clearance in black African men despite lower hepatic fat and similar whole-body and hepatic insulin sensitivity to their white European counterparts. It is increasingly recognised that type 2 diabetes is a heterogenous disease, where different aetiological components may have an impact on progression rates and choice of therapeutic strategy [67]. Ethnic disparities in treatment response have already been recognised [68] and may be explained, at least in part, by inherent physiological variations. In healthy black African men, the lack of association of endogenous insulin clearance with either intrahepatic lipid or insulin sensitivity supports the hypothesis that low insulin clearance is a primary phenomenon in this ethnic group. Such a phenomenon warrants further exploration, as it may offer novel therapeutic targets for the treatment and prevention of diabetes. Both the determinants of low insulin clearance and its role in the high risk of metabolic dysfunction in African ethnic populations remain to be elucidated.

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Statement of Data Availability

Data are available from the authors (LMG) on request.

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Contribution statement

L.M.G. formulated the research question and designed the study, supervised data collection and interpretation. S.A.A. formulated the research question and designed the study. J.L.P. formulated the research question, designed the study, and provided statistical advice. A.M.U. formulated the research question and designed the study. RCB supervised modelling analysis and contributed to the interpretation. O.B. supervised data collection, performed the metabolic assessments and undertook data analysis. FSM supervised data collection and undertook data analysis. MS performed metabolic assessments and contributed to the interpretation. M.L. supervised data collection, performed the metabolic assessments, undertook data analysis and interpretation and drafted the manuscript. O.H. undertook MRI data analysis. G.C.E. coordinated MRI data acquisition. LB undertook modelling analysis. All authors contributed to the intellectual content and reviewed the final version of the submitted manuscript.

LMG is the guarantor of this work, had full access to all the data and takes full responsibility for the integrity of the data and the accuracy of data analysis

Conflict of interest statement

SAA has served on advisory boards for NovoNordisk, Medtronic and Roche.
The other authors declare no conflict of interest.

Legends

Table 1: Clinical characteristics of study participants.

Table 2: Metabolic parameters of insulin secretion and insulin clearance

Figure 1

a: Plasma insulin response by ethnic group during the hyperglycaemic clamp.

b: Plasma C-peptide response by ethnic group during the hyperglycaemic clamp.

Figure 2

a: Associations between endogenous insulin clearance and whole-body insulin sensitivity (M value).

b: Associations between endogenous insulin clearance and whole-body insulin sensitivity (M/I).

c: Associations between endogenous insulin clearance and hepatic insulin sensitivity (% suppression EGP)

d: Associations between average endogenous insulin clearance and MCRI

e. Associations between endogenous insulin clearance and intrahepatic lipid (IHL)

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Table 1: **Clinical characteristics of study participants.**

	BA (n=23)	***WE (n=23)***	***p value***
Age (years)	30.7 (12.0)	35.9 (13.9)	0.18
Weight (kg)	84.1 (14.6)	86.5 (16.5)	0.60
BMI (kg m^{-2})	26.7 (3.6)	26.5 (4.6)	0.86
Waist circumference (cm)	87.5 (9.3)	93.8 (14.6)	0.09
Fasting glucose (mmol L^{-1})	5.25 (0.4)	5.20 (0.4)	0.51
HbA_{1c} IFCC (mmol/mol)	37.0 (5.3)	35.9 (2.9)	0.37
HbA_{1c} DCCT (%)	5.54 (0.48)	5.44 (0.24)	0.38
Systolic blood pressure (mmHg)	123.1 (12.3)	121.9 (9.1)	0.70
Diastolic blood pressure (mmHg)	70.7 (11.5)	71.1 (8.2)	0.88
LDL-cholesterol (mmol L^{-1})	2.66 (0.87)	2.99 (0.82)	0.19
HDL-cholesterol (mmol L^{-1})	1.30 (0.42)	1.27 (0.31)	0.75
Total cholesterol (mmol L^{-1})	4.27 (1.06)	4.76 (1.05)	0.13
Triglycerides (mmol L^{-1})	0.68 (0.25)	1.10 (0.56)	0.003

Data presented as mean (SD). Differences between the two ethnic groups determined using independent sample Student's *t* test. Fasting glucose values taken at screening visit. Abbreviations: BA black African; DCCT Diabetes Control and Complications Trial; HbA_{1c} glycated haemoglobin; HDL high density lipoprotein; IFCC International Federation of Clinical Chemistry; LDL low density lipoprotein; WE white European.

Table 2: Metabolic parameters of insulin secretion and insulin clearance

	BA (n=23)	WE (n=23)	Mean difference or ratio of geometric mean (95% CI)	*p* value
Fasting plasma glucose (mmol L^{-1})	5.26 (0.35)	5.19 (0.32)	-0.07 (-0.28, 0.13)	0.460
Fasting plasma C-peptide (nmol L^{-1}) †	0.54 (0.47, 0.62)	0.61 (0.50, 0.76)	1.14 (0.89, 1.47)	0.281
Fasting plasma insulin (pmol L^{-1}) †	46.2 (38.6, 55.3)	39.4 (30.2, 51.6)	0.85 (0.62, 1.17)	0.314
$iAUC_{c\text{-}pep}$ 0-10 mins † (nmol L^{-1} x min)	8.45 (6.17, 11.6)	6.88 (5.83, 8.3)	0.81 (0.58, 1.15)	0.240
$iAUC_{c\text{-}pep}$ 10-120 mins (nmol L^{-1} x min)	242.1 (109.9)	213.2 (60.4)	-28.9 (-82.0, 24.3)	0.277
$iAUC_{ins}$ 0-10 mins † (pmol L^{-1} x min)	2.46 (1.85, 3.28) $x10^3$	1.75 (1.5, 2.1) $x10^3$	0.71 (0.51, 0.98)	0.040
$iAUC_{ins}$ 10-120 mins † (pmol L^{-1} x min)	48.1 (35.5, 65.2) $x10^3$	29.9 (23.3, 38.4) $x10^3$	0.62 (0.42, 0.91)	0.017
AUC_{ISR} over 120 mins (pmol m^{-2} BSA x min)	58.9 (24.0) $x10^3$	54.4 (16.9) $x10^3$	-4.50 (-17.1, 8.14)	0.477
σ^1 † (pmol m^{-2} BSA)/ (mmol L^{-1} min^{-1})	692.9 (505.9, 948.9)	550.5 (464.9, 651.8)	0.80 (0.56, 1.13)	0.190
σ^2 (pmol min^{-1} m^2 BSA)/ (mmol L^{-1})	49.8 (20.1)	47.1 (18.8)	-2.67 (-14.4, 9.07)	0.649
Average (endogenous) insulin clearance (mL m^{-2} BSA min^{-1})	771.6 (227.8)	1381 (534.3)	609.5 (349.5, 869.6)	<0.001
MCRI (mL m^{-2} BSA min^{-1})	482.8 (70.6)	530.7 (78.7)	47.9 (1.6, 94.3)	0.043
Basal EGP (µmol kg^{-1} FFM min^{-1})	4.69 (2.39)	4.04 (2.29)	-0.65 (-2.13,0.83)	0.377
% suppression EGP ‡	65.7 (16.1)	69.8 (17.7)	4.15 (-6.5, 14.8)	0.437
% IHL §	3.78 (1.13)	6.08 (5.04)	2.29 (0.06, 4.52)	0.044
M value ‡ mg kg^{-1} FFM min^{-1}	9.65 (2.32)	9.51 (3.86)	-0.14 (-2.08, 1.81)	0.89
M/I ‡ (mg kg^{-1} FFM min^{-1})/ (pmol L^{-1})	0.0171 (0.0059)	0.0189 (0.0094)	0.00184 (-0.0030, 0.0066)	0.44

Data presented as mean (SD) or geometric mean (95% CI) for log transformed data (†). Differences between the two ethnic groups were determined using independent samples t-tests. Fating plasma glucose levels from hyperglycaemic clamp visit. ‡ Previously reported data [26] § Previously reported data [30]

Abbreviations: BA, black African; BSA: Body surface area; EGP, Endogenous glucose production; FFM, Fat free mass. IHL, Intrahepatic lipid; WE, white European; iAUC, incremental area under the curve; AUC(ISR), area under the curve of insulin secretion rate over 120 minutes; MCRI, metabolic clearance rate of insulin.

Figure 1a: Plasma insulin response by ethnic group during the hyperglycaemic clamp. BA = black African, WE = white European. Data shown are mean and SD.

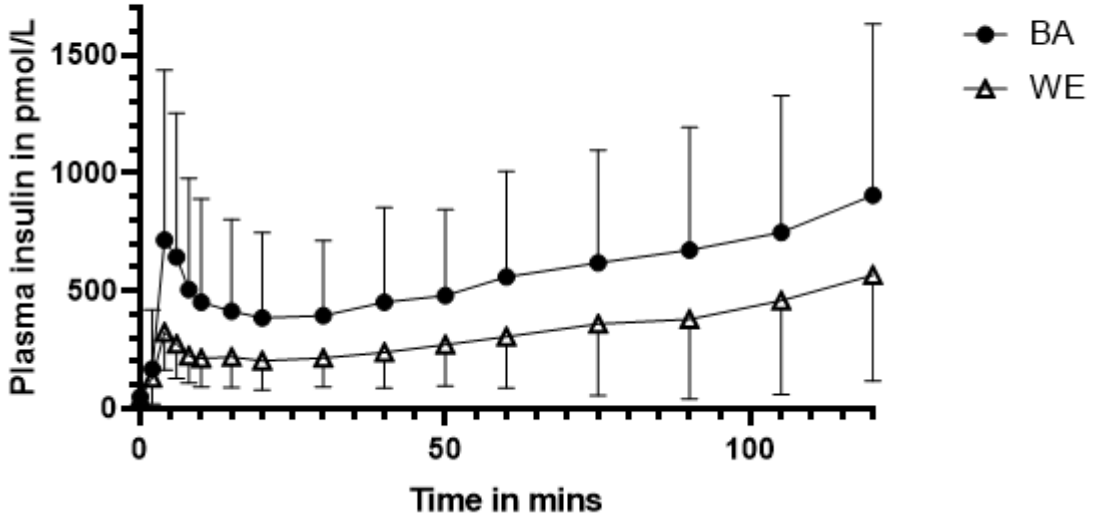


Figure 1b: Plasma C-peptide response by ethnic group during the hyperglycaemic clamp. BA = black African, WE = white European. Data shown are mean and SD.

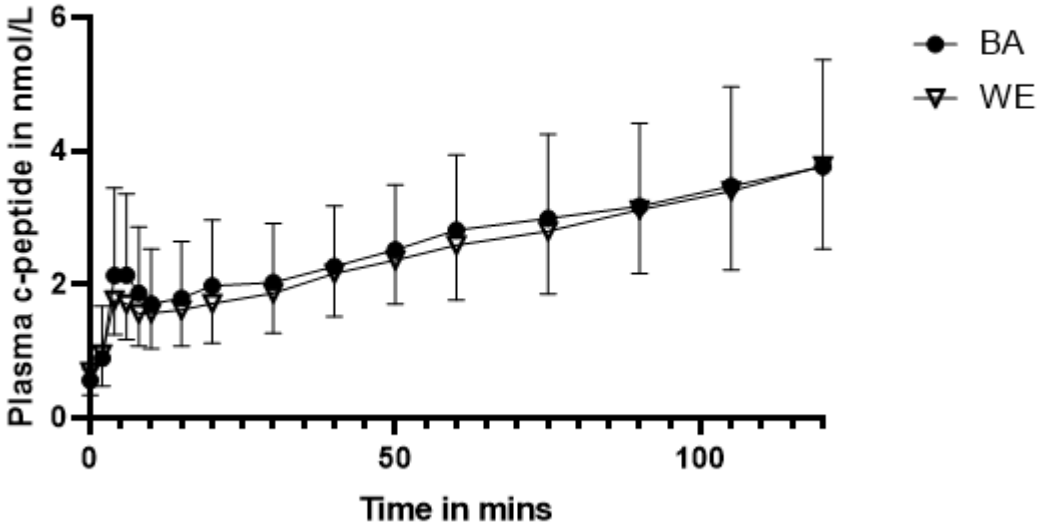


Figure 2a: Associations between average insulin clearance and whole-body insulin sensitivity (M value) in white European (WE) and black African (BA) men. Data presented using the Pearson correlation coefficient: BA: r= 0.051, *p*=0.837; WE: r=0.691, *p*=0.001. Interaction by ethnicity was assessed using linear multiple regression. FFM, fat free mass.

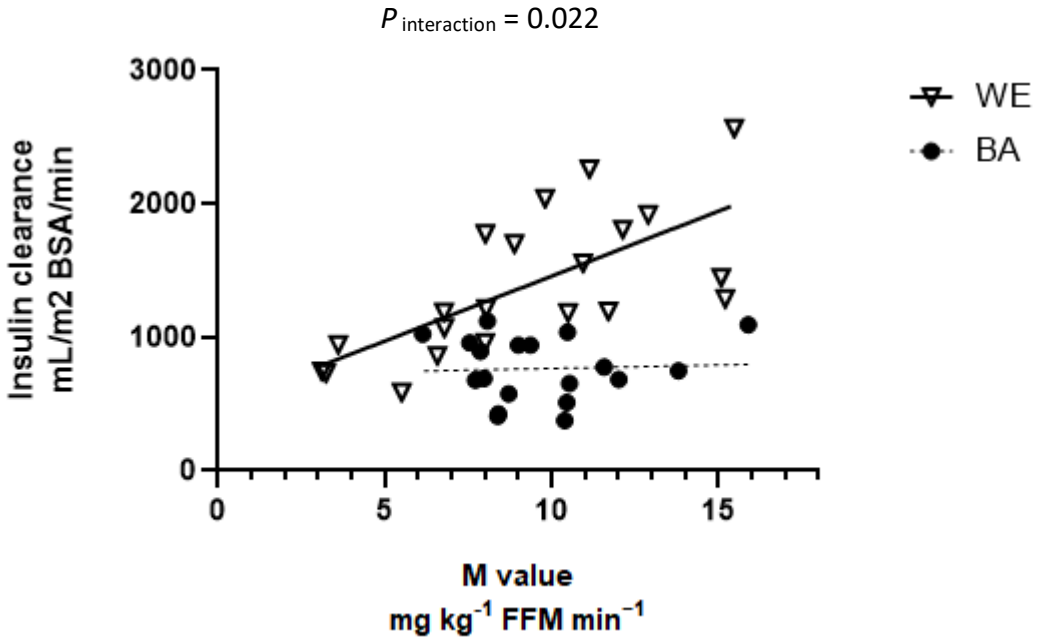


Figure 2b: Associations between average insulin clearance and whole-body insulin sensitivity (M/I) in white European (WE) and black African (BA) men. Data presented using the Pearson correlation coefficient: BA: r= 0.179, *p*=0.464; WE: r=0.697, *p*<0.001. Interaction by ethnicity was assessed using linear multiple regression.

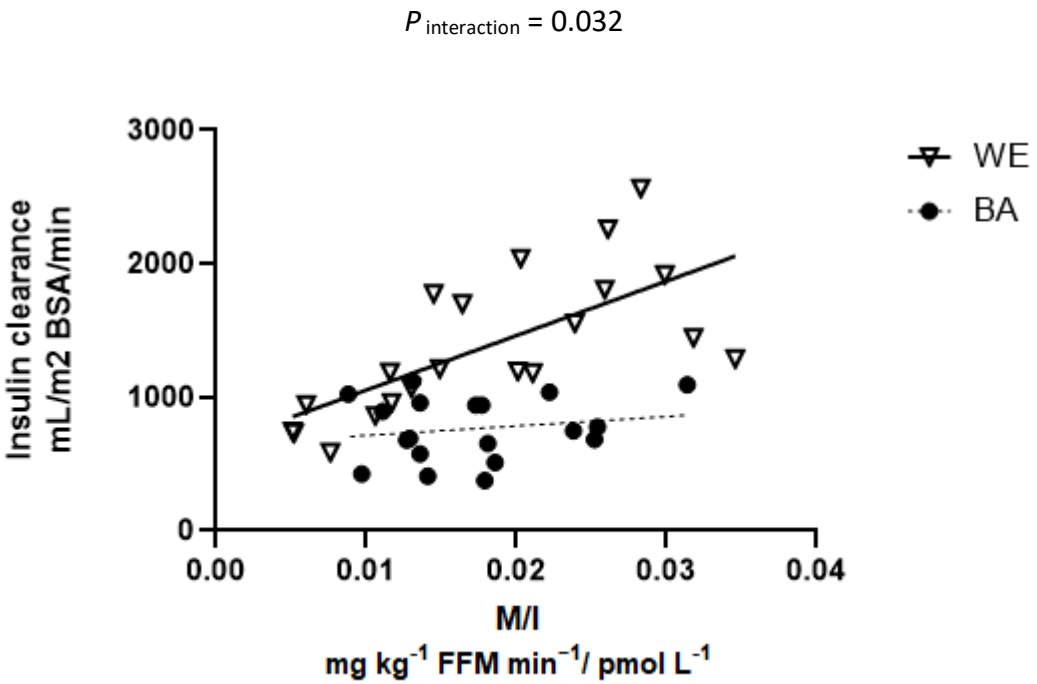


Figure 2c: Associations between average insulin clearance and hepatic insulin sensitivity (% suppression EGP) in white European (WE) and black African (BA) men. Data presented using the Pearson correlation coefficient: BA: r= -0.169, *p*=0.488; WE: r=0.417, *p*=0.068. Interaction by ethnicity was assessed using linear multiple regression.

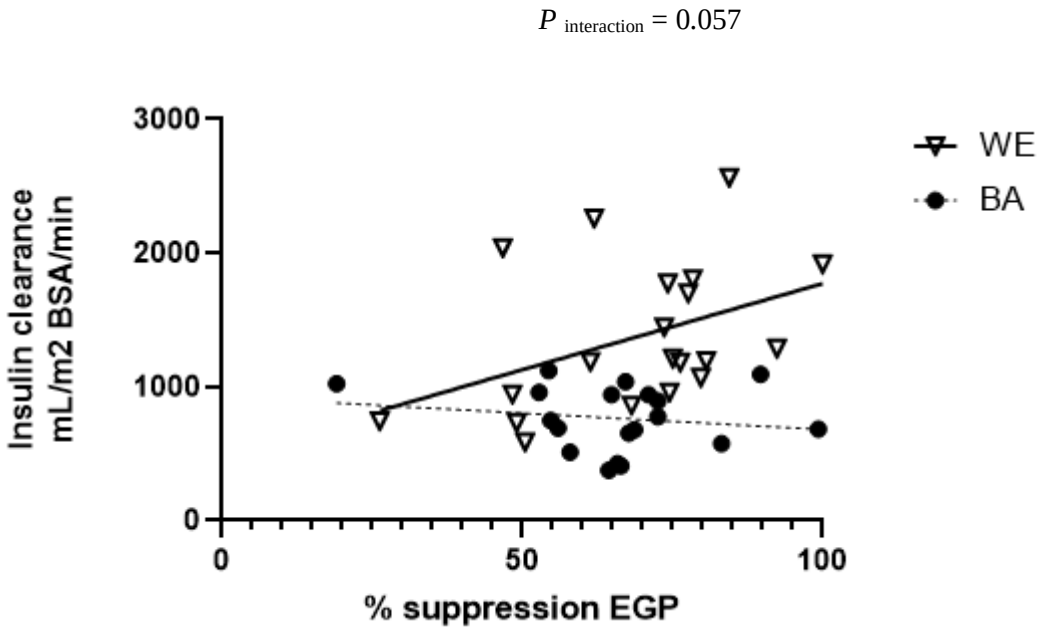


Figure 2d: Associations between average endogenous insulin clearance (measured during hyperglycaemic clamp) and metabolic clearance rate of (exogenous) insulin (MCRI, measured during hyperinsulinaemic-euglycaemic clamp) in white European (WE) and black African (BA) men. Data presented using the Pearson correlation coefficient: r=0.298, *p*=0.215 ; WE: r=0.661, *p*=0.001. Interaction by ethnicity was assessed using linear multiple regression.

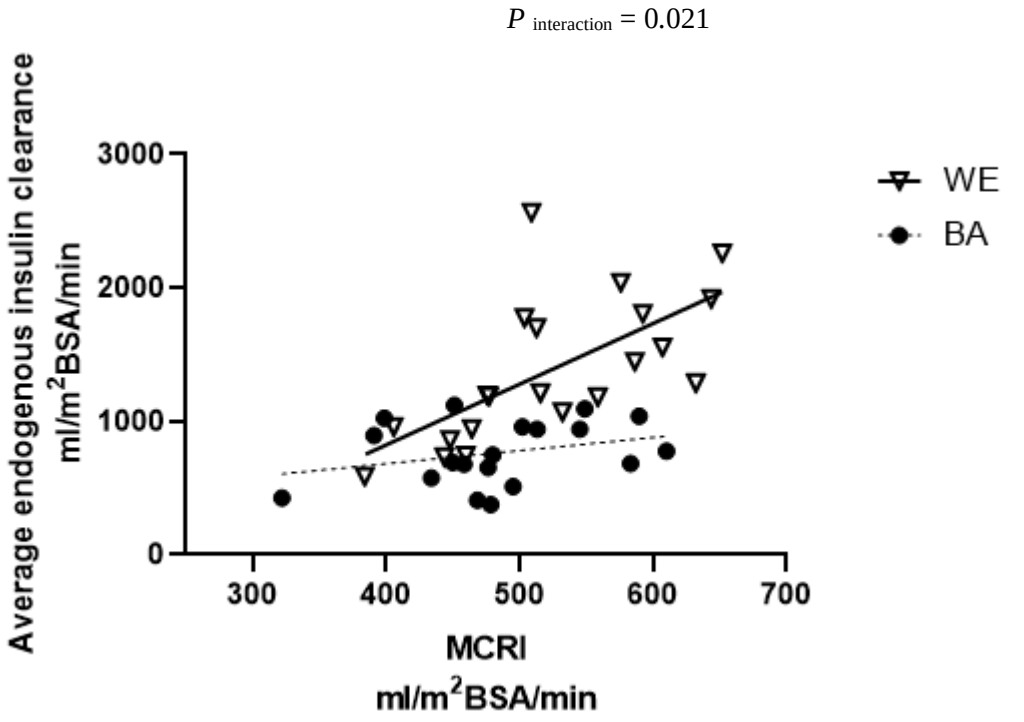
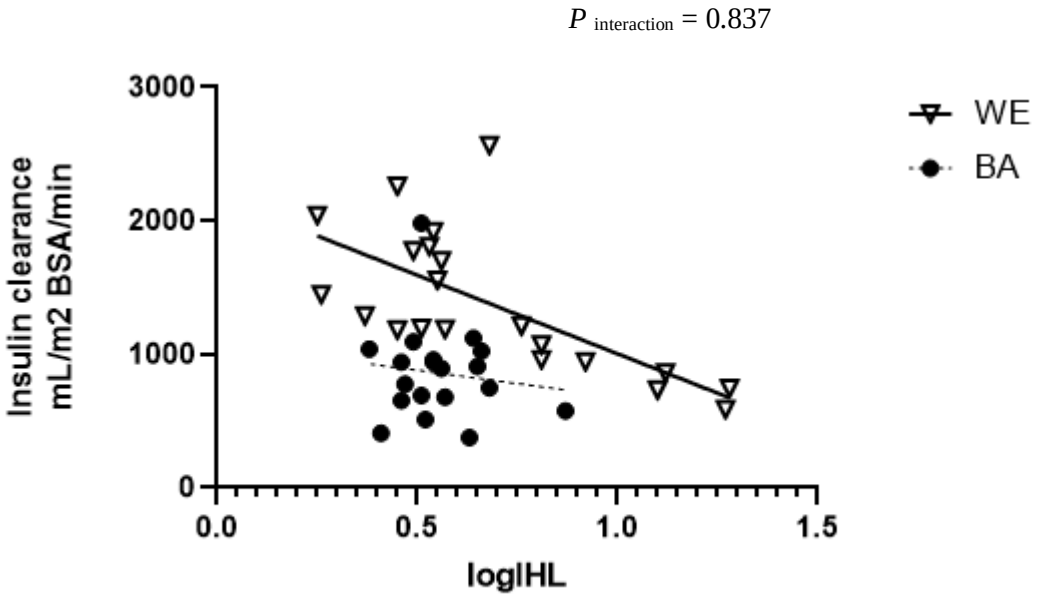


Figure 2e: Associations between average insulin clearance and intrahepatic lipid (IHL) in white European (WE) and black African (BA) men. Data presented using the Pearson correlation coefficient: BA: r= -0.134, *p* =0.584; WE: r= -0.674, *p*= 0.001. Interaction by ethnicity was assessed using linear multiple regression.



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